



## Effects of Different Levels of Dietary Energy and Nutrient Density during the Pre-Peak and Peak Periods on Egg Quality in Hy-Line Brown Laying Hens

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**ABSTRACT** In this study, we investigated the effects of feeding diets with different levels of energy and nutrient density on the egg quality of laying hens during the pre-peak and peak periods. A total of 192 (Hy-line brown) laying hens were used in a 15-week trial. The hens were randomly allotted to one of four treatments, each with four replicates (12 hens per replication). We assessed the effects of four level of dietary energy (2,710, 2,850, 2,870 and 2,890 kcal/kg) and three levels of nutrient density (Methionine + Cysteine: 0.56%, 0.85%, 0.80% and Crude Protein: 14.5%, 19%, 18%). Differences in the energy and nutrient density contents of diets showed no significant effect ( $P>0.05$ ) on the average daily gain, average daily feed intake, feed conversion ratio, egg weight, or egg production of hens during the pre-peak and peak periods. However, hens subjected to 2,890 kcal/kg during the pre-peak period were found to lay eggs with significantly thicker shells, and yolk color was found to be significant enhanced in hens fed this diet during the pre-and peak periods. In contrast, we detected no significant effects of dietary energy or nutrient density on the Haugh unit or eggshell strength. In summary, increasing the energy level of diets from 2,710 to 2,890 kcal/kg was found to have positive effects on the shell thickness and yolk color of eggs produced by laying hens.

(Key words: laying hens, egg weight, egg production, egg quality)

## INTRODUCTION

Over the past decades, nutritional supplements were added in animal meals to enhances the quality and quantity of animal products. The formulation of poultry feed is based on the concept that poultry tend to meet their energy needs, assuming that the diet is adequate in all other essential nutrients. Owing to this concept, the crude proteins and amino acids added to the poultry diet should be proportionate to meet the metabolizable energy concentration (NRC, 1984). As of the assumption that laying hens require less high-energy diet meaning that they will only eat to meet their energy requirement, and since the ingredients in high-energy diet include corn grain, vegetable oil, etc., which are quite expensive, often low-energy feeds are also fed. Though low-energy diets are fed and the energy requirement for laying hens may not seem sufficient, hens can regulate their feed intake rate to maintain their energy requirement (Harms

et al., 2000; Leeson et al., 2001; Wu et al., 2005, 2007). Birds of low body weight, and low inherent feed intake efficiently meet their energy requirements by adjusting to their feed intake in place of changing diet nutrient density, therefore by increasing their feed intake in response to marginal levels of first limiting amino acids, independent of diet energy level (Keshavarz and Nakajima, 1995).

Wu et al. (2005b) suggested that increasing the dietary proteins and amino acids in diet may prevent the interfering effect of decreased nutrient intake on egg weight so that the true effect of increasing dietary energy on egg weight could be determined. The better understanding of the effect of increasing both dietary energy and other nutrients (amino acids, Ca, and available P) may help egg producers to optimize early egg weight to improve profits, especially when a large egg price spread due to egg size exists. The main problem that concerns the poultry producers are small egg size in young hens during peak production, and if egg weight

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can be improved, poultry producers will be able to improve profits, depending upon egg price, egg size, and ingredient prices. Therefore, the objective of our present study was to investigate the effects of varied energy and nutrients density diets during the pre-peak and peak period on egg quality in laying hens.

## MATERIALS AND METHODS

The experiment protocol was reviewed and approved (DK-1-2012) by the Animal Care and Use Committee of Dankook University (Cheonan, Republic of Korea).

### 1. Experimental Design, Birds, and Housing

A total of 192 (Hy-line brown) laying hens were used in a 15-week trial as pre-peak and peak period (growing period –0-7 weeks, pre-peak period –7-13 weeks and peak period

–13-15 weeks). Laying hens were randomly allotted to 1 of 4 treatments with 4 replications (12 hens per replication). The four level of dietary energy supplements were: 2,710, 2,850, 2,870 and 2,890 kcal/kg, and the three different level of nutrient density were: Methionine + Cysteine, %: 0.56, 0.85, 0.80 and Crude Protein, %: 14.5, 19, 18. From weeks 1-7 laying hens were fed with: CON (basal diet), TRT1, TRT2, and TRT3 –CP: 14.5% (M+C: 0.56)/ ME: 2,710, whereas from weeks 7-13 hens were offered to had : CON, CP: 14.5% (M+C: 0.56)/ ME: 2,710; TRT1, CP: 19% (M+C: 0.85)/ ME: 2,850; TRT2, CP: 19% (M+C: 0.85)/ ME: 2,870; TRT3, CP: 19% (M+C: 0.85)/ ME: 2,890, and from weeks –13-15 –CON, TRT1, TRT2, and TRT3 –CP: 18% (M+C: 0.80)/ ME: 2,850. Ingredients and nutrient composition of experimental diets are shown in Table 1. All nutrients diets were formulated to meet or exceed the recommendation of NRC (1994). The laying hens were individually caged and provided with free access to water and feed by nipple

**Table 1.** Ingredient composition of experimental diets as-fed basis

Raw material	CP: 14.5% (M+C: 0.56)/ ME: 2,710	CP: 19% (M+C: 0.85)/ME:2,850 kcal/kg)	CP: 19% (M+C: 0.85)/ME:2,870 kcal/kg)	CP: 19% (M+C: 0.85)/ME:2,890 kcal/kg)	CP: 18% (M+C: 0.80)/ ME: 2,850kcal/kg)
Corn (Non-GMO)	51.01	49.01	48.53	48.06	50.61
Wheat	6	4	4	4	5
Soy (full fat)	3	8	8	8	8
Wheat Bran	16				
Soy bean meal (Non-GMO)	3.42	8.46	8.54	8.63	5.19
Canola meal	1	1	1	1	1
Corn gluten meal (Non-GMO)	1.72	7	7	7	7
Sesame Meal	4.5	4.5	4.5	4.5	4.5
DDGS (distiller's dried grains with soluble)- Corn	4	4	4	4	4
Palm kernel meal	3				1.47
Tallow	0.5	0.86	1.26	1.66	0.5
Molasses (Cane)	0.5	0.5	0.5	0.5	0.5
Limestone	3.45	10.12	10.12	10.11	9.95
MDCP (Mono dicalcium Phosphate)	0.97	1.1	1.1	1.1	0.75
Salt	0.17	0.1	0.1	0.1	0.02
NaHCO <sub>3</sub> (Sodium bicarbonate)	0.21	0.28	0.28	0.27	0.4

**Table 1.** Continued

Raw Material	CP: 14.5% (M+C: 0.56)/ ME: 2,710	CP: 19% (M+C: 0.85)/ME:2,850 kcal/kg)	CP: 19% (M+C: 0.85)/ME:2,870 kcal/kg)	CP: 19% (M+C: 0.85)/ME:2,890 kcal/kg)	CP: 18% (M+C: 0.80)/ ME: 2,850kcal/kg)
Methionine (99%)	0.01	0.18	0.18	0.18	0.15
Lysine (50%)	0.24	0.46	0.46	0.46	0.51
Threonine (98.5%)		0.02	0.02	0.02	0.02
Tryptophan (20%)		0.09	0.09	0.09	0.11
Vitamin premix	0.12	0.12	0.12	0.12	0.12
Choline (50%)	0.08	0.1	0.1	0.1	0.1
Mineral premix	0.1	0.1	0.1	0.1	0.1
<b>Calculated value</b>					
Moisture (%)	11.55	10.01	9.95	9.9	10.06
Crude protein (%)	14.5	19	19	19	18
Crude fat (%)	4.6	5.29	5.67	6.05	5.07
Crude fiber (%)	4.24	2.8	2.79	2.79	2.87
Crude ash (%)	7.72	14.49	14.49	14.49	13.94
ME (kcal/kg)	2,710	2,850	2,870	2,890	2,850
Ca (%)	1.6	4.12	4.12	4.12	4
Available P (%)	0.45	0.45	0.45	0.45	0.39
Methionine + Cysteine (%)	0.56	0.85	0.85	0.85	0.8

<sup>1</sup> Provided per kilograms of diet: vitamin A, 10,800 IU; vitamin D<sub>3</sub>, 4,000 IU; vitamin E, 40 IU; vitamin K<sub>3</sub>, 4 mg; vitamin B<sub>1</sub>, 6 mg; vitamin B<sub>2</sub>, 12 mg; vitamin B<sub>6</sub>, 6 mg; vitamin B<sub>12</sub>, 0.05 mg; biotin, 0.2 mg; folic acid, 2 mg; niacin, 50 mg; D-calcium pantothenate, 25 mg.

<sup>2</sup> Provided per kg diet: Fe, 100 mg as ferrous sulfate; Cu, 17 mg as copper sulfate; Mn, 17 mg as manganese oxide; Zn, 100 mg as zinc oxide; I, 0.5 mg as potassium iodide; and Se, 0.3 mg as sodium selenite.

drinkers and feeders. Room temperature was maintained at 21±1°C and had a daily lighting schedule of sixteen hours light and eight hours dark.

## 2. Sampling and Laboratory Analysis

Body weight measurement was done at initial, week 11, and week 15. Average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated and daily records of egg production was maintained. The production of egg was expressed as an average hen-day production. Also, the quality of the egg was checked alternative week from week 9 to 15. A total of 120 eggs (30-eggs each treatment) from each treatment was

randomly selected and the quality of eggs were analyzed on the same day. The egg weight, shell strength, yolk, and Haugh Unit were measured using an egg multi tester (Touhoku Rhythm Co. Ltd., Tokyo, Japan). Eggshell breaking strength was determined with the eggshell force gauge (model II, Robotmation Co., Ltd., Tokyo, Japan). A dial pipe gauge (Ozaki MFG. Co., Ltd., Tokyo, Japan) was used to measure eggshell thickness, which was determined based on the average thickness of the rounded end, pointed end, and the middle of the egg, excluding the inner membrane.

## 3. Statistical Analysis

All the data were analyzed using the GLM procedure of

the SAS program SAS (Inst. Inc., Cary, NC) Software package (2000). The Duncan's multiple test was performed to determine the significant difference. The data were expressed as the standard error of the mean (SEM), and *P* values <0.05 were considered as statistical significance and *P* values <0.10 as trend.

## RESULTS AND DISCUSSIONS

The effects of variation in energy and nutrient density of the experimental diets on growth performance and egg production in laying hens is shown in Table 2. No significant difference was observed on egg weight with the variation in energy and nutrient density diets during pre-peak and peak period (*P*>0.05). Similarly, Wu et al. (2005) reported that egg weight did not have any significant effect with an increasing level of dietary energy from 2,877 to 2,956 kcal of ME/kg. However, earlier studies reported inconsistent results, that

increasing dietary fat or dietary energy had significantly improved early egg weight (Keshavarz, 1995; Keshavarz and Nakajima, 1995; Grobas et al., 1999; Harms et al., 2000; Bohnsack et al., 2002; Sohail et al., 2003; Wu et al., 2005b). Wu et al. (2005b) demonstrated that an ideal dietary energy: protein is required for optimal performance of laying hens thereby having an impact on egg weight. DePersio et al. (2015) in their study indicated that feeding energy and nutrient rich diet to hens will increase egg production, egg weight, and BW. In the present study, the different levels of energy and nutrient density diets did not have any significant effects (*P*>0.05) on BW, ADFI, FCR, and egg production during pre-peak and peak period. However, except early production cycle there were no significant results observed with the increasing energy and nutrient density diets. Rao et al. (2014) stated that increased feed intake occurred between 21 to 72 weeks of age, when the birds were fed a diet containing 2,399 vs. 2,550 vs. 2,700 kcal/kg ME, respectively.

**Table 2.** The effects of different level of energy and nutrient density on growth performance and egg production in laying hens<sup>1</sup>

Items	CON	TRT1	TRT2	TRT3	SEM <sup>2</sup>	<i>P</i> value
Growing						
BW (g)	1,583	1,616	1,655	1,641	0.52	0.6023
ADFI (g)	80.92	81.14	80.13	80.11	0.59	0.5436
Pre-peak (11 week)						
BW (g)	1,809	1,788	1,813	1,805	1.30	0.6023
ADFI (g)	89.64	92.49	89.53	91.82	1.06	0.1812
Egg production, %	70.07	67.43	68.89	62.15	3.67	0.6037
Egg weight, g	48.23	49.95	48.63	50.56	1.22	0.9596
FCR	3.053	3.368	3.150	3.094	0.173	0.4081
Peak (15 week)						
BW (g)	1,889	1,840	1,864	1,887	1.35	0.6023
ADFI (g)	96.50	97.50	97.39	97.92	0.64	0.4111
Egg production (%)	90.72	93.84	93.37	89.77	2.52	0.6037
Egg weight (g)	53.56	53.93	53.89	53.94	0.58	0.4596
FCR	1.898	1.840	1.879	1.938	0.056	0.6782

<sup>1</sup> Abbreviation: 1-7 weeks - CON, TRT1, TRT2, TRT3, CP: 14.5% (M+C: 0.56)/ ME: 2,710; 7-13 weeks - CON, CP: 14.5% (M+C: 0.56)/ ME: 2,710; TRT1, CP: 19% (M+C: 0.85)/ ME: 2,850; TRT2, CP: 19% (M+C: 0.85)/ ME: 2,870; TRT3, CP: 19% (M+C: 0.85)/ ME: 2,890; 13-15 weeks - CON, TRT1, TRT2, TRT3, CP: 18% (M+C: 0.80)/ ME: 2,850.

<sup>2</sup> Standard error of means.

Moreover, Jalal et al. (2007) showed that the feed intake in Hy-Line W-36 hens was not improved when fed a low-energy diet (2,810 vs. 2,900 kcal ME/kg). During early stages of lay cycle, the nutrient requirements for younger and older hen differs, where younger ones require higher nutrient concentrations than older ones due to low feed intake (NRC, 1994). Harms et al. (2000) and Wu et al. (2005) also indicated that egg production was not affected by dietary energy. DePersio et al. (2015) studies showed that 85% treatments were not effective in young birds to reach production in the first phase, furthermore, laying hen does not have the capacity to increase its feed intake when fed a diet of low nutrient-density. In this study, the reason behind

this problem might be due to the variation in low nutrient density, which can result in low feed intake and low egg production in the early lay cycle.

Previously, Zhang and Kim (2013) and Kang et al. (2018) reported that the eggshell thickness, eggshell strength, yolk color, and Haugh unit were not affected by inclusion of energy and nutrient density diets (2,700 or 2,800 kcal/kg ME) during 0 to 6 weeks. Similarly, Ribeiro et al. (2014) did not find any influence of dietary energy level 2,700 to 3,000 kcal/kg on eggshell percentage, yolk color, HU of layer eggs. The effects of different levels of energy and nutrient density on egg quality in laying hens is shown in Table 3. The quality parameter which usually depends on retailer's desire

**Table 3.** The effects of different level of energy and nutrient density on egg quality in laying hens<sup>1</sup>

Items	CON	TRT1	TRT2	TRT3	SEM <sup>2</sup>	<i>P</i> value <sup>3</sup>
Pre-peak (Week 9)						
Haugh unit	70.85	61.04	67.38	64.76	4.84	0.5375
Yolk color	10.31	10.13	10.44	10.13	0.17	0.4853
Eggshell strength, kg/cm <sup>2</sup>	5.20	4.56	4.70	4.71	0.20	0.1268
Eggshell thickness, mm	35.33 <sup>b</sup>	38.60 <sup>a</sup>	38.81 <sup>a</sup>	39.04 <sup>a</sup>	0.45	0.0003
Pre-peak (Week 11)						
Haugh unit	77.39	76.90	78.78	82.73	6.40	0.9160
Yolk color	9.75 <sup>b</sup>	9.88 <sup>ab</sup>	10.13 <sup>a</sup>	10.19 <sup>a</sup>	0.11	0.0383
Eggshell strength, kg/cm <sup>2</sup>	4.67	5.21	4.76	5.19	0.19	0.1051
Eggshell thickness, mm	37.73 <sup>b</sup>	37.96 <sup>b</sup>	44.88 <sup>a</sup>	46.69 <sup>a</sup>	0.64	<0.0001
Peak (Week 13)						
Haugh unit	66.84	73.97	77.05	77.07	4.35	0.3080
Yolk color	9.10	8.70	8.95	8.95	0.20	0.5611
Eggshell strength, kg/cm <sup>2</sup>	5.35	5.49	5.40	5.07	0.18	0.3688
Eggshell thickness, mm	38.38	38.40	38.38	38.58	0.30	0.2001
Peak (Week 15)						
Haugh unit	77.59	83.70	80.74	78.47	4.60	0.7884
Yolk color	8.50	8.75	8.25	8.50	0.15	0.1336
Eggshell strength, kg/cm <sup>2</sup>	4.59	4.65	4.70	4.51	0.20	0.9125
Eggshell thickness, mm	38.88	38.83	39.25	40.62	0.48	0.1856

<sup>1</sup> Abbreviation: 1-7 weeks - CON, TRT1, TRT2, TRT3, CP: 14.5% (M+C: 0.56)/ ME: 2,710; 7-13 weeks - CON, CP: 14.5% (M+C: 0.56)/ ME: 2,710; TRT1, CP: 19% (M+C: 0.85)/ ME: 2,850; TRT2, CP: 19% (M+C: 0.85)/ ME: 2,870; TRT3, CP: 19% (M+C: 0.85)/ ME: 2,890; 13-15 weeks - CON, TRT1, TRT2, TRT3, CP: 18% (M+C: 0.80)/ ME: 2,850.

<sup>2</sup> Standard error of means.

<sup>3</sup> Means in the same row with different superscripts differ ( $P < 0.05$ ).

in eggs is the color of the yolk, because consumers associate this parameter with high nutritional value and vitamin content (Galobart et al., 2004; Zhang and Kim, 2011). In the current study, the yolk color showed significant improvement ( $P=0.0383$ ) in treatment 3 at the pre-peak period (week 11). Silva et al. (2007) found that, laying hen when fed with diet containing high oil content tend to have higher yolk color values. In the present study, the supplementation of energy (2,710 to 2,890 kcal/kg) and nutrient density into the diet of laying hens significantly increased eggshell thickness in 2,890 kcal/kg during the pre-peak period (week 9 and 11). Junqueira et al. (2006) stated that eggshell percentage had significant effect by the addition of different level of energy in diets. The results stated by Grobas et al. (1999) showed no effect in egg shell quality in 22 to 75 weeks age laying hens in different dietary energy (2,700 to 3,000 kcal of ME/kg) levels. In contrast, Mendonca and Lima (1999) evaluated that laying hens early in the second productive cycle which are fed with a diet of 14.5% protein than those from birds fed diets with 16.5% protein found better eggshell quality. Haugh unit is a measure of the freshness of an egg. In a study conducted by Junqueira et al. (2006) failed to observe any significant effects on Haugh units of eggs, ( $P>0.05$ ) obtained from layers fed different levels of energy. (2,850; 2,950, and 3,050 kcal of ME/kg) and protein (16, 18, and 20% CP). Similarly, Zimmermann and Andrews (1987) compared diets with 2 levels of metabolizable energy (3,100 or 2,920 kcal of ME/kg) and 2 levels of protein (14.6 and 15.5%) on performances of laying hens and did not find any effect on Haugh units. In our study, Haugh unit was not affected in the pre-peak period and peak period by feeding the birds with diets containing different levels of energy and protein. In contrast, Silva et al. (2007) found a positive quadratic effect in Haugh units as dietary metabolizable energy intake increased, whereas Wu et al. (2005, 2007) reported a reduction in HU values as dietary metabolizable energy concentration increased. The contradiction between the various study results might be due to the age of hens, composition of dietary energy and nutrients level.

## SUMMARY

In conclusion, eggshell thickness and yolk color had positive effects due to the increasing energy level from 2,710 to 2,890 kcal/kg during the pre-peak period. The difference in the levels of energy and nutrient density in the diets failed to show significant effects on BW, ADFI, FCR, and egg production during pre-peak and peak period. Also, eggshell strength and HU had no effect by formulating the diets with varying energy, amino acids and protein levels. Among the four treatments, 2,890 kcal/kg was better than control and other treatments. Therefore, we conclude that an energy level that ranges between 2,710 to 2,890 kcal/kg would be potential to improve the quality of eggs.

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